Pyrazoles I. Synthesis of 4-Hydroxypyrazolo[3,4-*d*]-*v*-triazine A New Analog of Hypoxanthine

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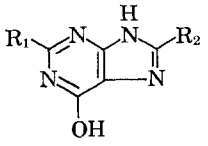
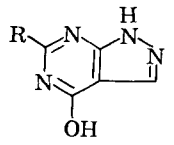
Treatment of 3-amino-4-pyrazolecarboxamide with nitrous acid yielded 3-diazo-4-pyrazolecarboxamide, which was cyclized to 4-hydroxypyrazolo[3,4-*d*]-*v*-triazine in aqueous solution. The latter is a structural analog of hypoxanthine as well as an aza analog of allopurinol.

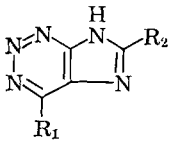
A STRUCTURAL ANALOG of hypoxanthine (Ia), 4-hydroxypyrazolo[3,4-*d*]pyrimidine (IIa, 4-HPP, allopurinol, Zyloprim), was synthesized and reported in 1956 (1, 2). Compound IIa has since been found to be effective against hyperuricemia associated with gout in man (3–11). It is remarkably well tolerated by patients, and serious side effects have not been encountered (12–16). Metabolic studies revealed that many compounds of this type are potent inhibitors of xanthine oxidase (4, 14, 17–23), the enzyme responsible for the oxidation of hypoxanthine (Ia) to xanthine (Ib) and to uric acid (Ic). This compound can, therefore, be used to reduce the hazard of uric acid nephropathy in patients with leukemias and lymphomas, in whom rapid lysis of cells is expected to occur (24). It has also been used to potentiate the therapeutic index of 6-mercaptopurine (17, 18).



I a, R_1, R_2 = H
b, R_1 = OH, R_2 = H
c, R_1, R_2 = OH



II a, R = H
b, R = OH



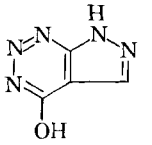
III a, R_1 = OH, R_2 = H
b, R_1 = NH_2, R_2 = H
c, R_1 = NH_2, R_2 = OH

Allopurinol itself is gradually metabolized *in vivo* by xanthine oxidase to 4,6-dihydroxypyrazolo[3,4-*d*]pyrimidine (25, 26) (IIb, isoxanthine, alloxanthine). Although IIb is still an inhibitor of xanthine oxidase, it is no longer as effective as allopurinol. Further metabolic oxidation of IIb to a uric acid-type analog is not feasible owing to the aza substituent in the original "purine-C_8" position.

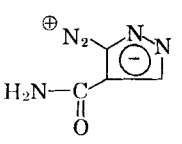
Some 2-azapurines (III, imidazo[4,5-*d*]-*v*-triazines) are also found to be inhibitors of xanthine oxidase (27). The most active one of this class, 2-azaadenine (IIIb), was reported to be rapidly attacked by this enzyme to form a somewhat less effective inhibitor, 8-hydroxy-2-azaadenine (27) (IIIc).

Since xanthine oxidase oxidizes purines at the 2, 6, or 8 position (28), synthesis of an aza analog of allopurinol (IIa), 4-hydroxypyrazolo[3,4-*d*]-*v*-triazine (IV) was proposed. Compound IV itself would not be subject to attack by xanthine oxidase, and thus could well be a better inhibitor of the enzyme. The fact that many 2-azapurines possess activity as inhibitors of neoplastic cells (29, 30) and of microorganisms (31) suggests that compounds such as IV might be useful as chemotherapeutic agents.

Treatment of 3-amino-4-pyrazolecarboxamide (1) with nitrous acid yielded a tan-colored compound which decomposed violently at *ca.* 165–170° on rapid heating. Although this product gave the correct elemental analysis for IV, a sharp, intense infrared absorption peak at the 4.5-μ region and a positive Bratton-Marshall test (32) indicated the presence of a diazo group. Hence the product was actually the uncyclized 3-diazo-4-pyrazolecarboxamide (V).



IV



V

Formation of a similar compound, 5-diazo-4-imidazolecarboxamide, from 5-amino-4-imidazolecarboxamide and nitrous acid has also been reported (33).

The desired cyclized compound, 4-hydroxypyrazolo[3,4-*d*]-*v*-triazine (IV, 4-HPT), can be readily obtained by treatment of V with dilute alkali or, less readily, with dilute acid. Compound IV did not give a positive Bratton-Marshall test (32), possessed no infrared absorption in the triple or cumulative double bond region. It can be recrystallized from water as a monohydrate.

Compound 4-HPT was tested as an inhibitor of xanthine oxidase from bovine milk. A 50% inhibition of the enzyme was observed by 4-HPT at a concentration of 7.4 μM in the presence of 8.1 μM of hypoxanthine as a substrate.

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EXPERIMENTAL[1]

3-Diazo-4-pyrazolecarboxamide (V)—To a finely powdered suspension of 35 Gm. (0.2 mole) of 3-amino-4-pyrazolecarboxamide hemisulfate (1) in 350 ml. of water cooled to 0° was added, with mechanical stirring, 16 Gm. (0.23 mole) of sodium nitrite dissolved in 60 ml. of water. The mixture was stirred for 3 hr. at 0° and the resulting tan solid was collected by filtration and washed successively with water, methanol, and ether. The yield after overnight air drying was 18 Gm. (66%), which, on rapid heating, decomposed violently with a sharp sound. The product, which is analytically pure, is surprisingly stable under ordinary storage conditions. It gave a positive Bratton-Marshall test (32) and exhibited a diazo absorption band at 4.5 μ. $\lambda_{max.}^{pH\ 1}$ 256 mμ (ϵ 6,000); $\lambda_{max.}^{pH\ 11}$ 311 mμ (ϵ 5,500).

Anal.—Calcd. for $C_4H_3N_5O$: C, 35.03; H, 2.20; N, 51.09. Found: C, 34.74; H, 2.36; N, 51.00.

4-Hydroxypyrazolo[3,4-*d*]-*v*-triazine Monohydrate (IV)—A white crystalline solid gradually separated on standing from the filtrate of the preceding experiment. The product was collected by filtration, washed with a small amount of cold water, and dried to yield 4 Gm. of IV. The compound, which is quite soluble in methanol, decomposed abruptly (but not as violently as V) at *ca.* 180° on rapid heating; slow heating did not cause it to decompose or melt up to 360°. The product is analytically pure but recrystallization from hot water changed the color of the solid from white to light yellow. The ultraviolet and infrared absorption spectra were, however, identical. Compound IV did not show a triple bond absorption peak and failed to yield a positive Bratton-Marshall test (32). $\lambda_{max.}^{pH\ 1}$ 270 mμ (ϵ 5,500); $\lambda_{max.}^{pH11}$ 312 mμ (ϵ 5,800).

Anal.—Calcd. for $C_4H_3N_5O \cdot H_2O$: C, 30.97; H, 3.25; N, 45.15; H_2O, 11.51. Found: C, 31.30; H, 3.53; N, 44.79; H_2O, 11.48.

The same product can also be obtained by the addition of 10 ml. of 10 *N* sodium hydroxide to the stirred suspension of 5 Gm. of V in 100 ml. of water. The resulting solution was allowed to stir for 40 min., filtered, and the filtrate acidified with dilute hydrochloric acid. Three and one-half grams of analytically pure light golden yellow plates were obtained.

[1] Melting points were taken on a Thomas-Hoover melting point apparatus. The infrared spectra were taken with a Perkin-Elmer infracord and the ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer.

REFERENCES

(1) Robins, R. K., *J. Am. Chem. Soc.*, **78**, 784(1956).
(2) Schmidt, P., and Durey, J., *Helv. Chim. Acta*, **39**, 986(1956).
(3) Feigelson, P., Davidson, J. D., and Robins, R. K., *J. Biol. Chem.*, **226**, 993(1957).
(4) Rundles, R. W., Wyngaarden, J. B., Hitchings, G. H., Elion, G. B., and Wilberman, H. R., *Trans. Assoc. Am. Physicians*, **76**, 126(1963).
(5) Klinenberg, J. R., Goldfinger, S., Miller, J., and Seegmiller, J. E., *Arthritis Rheumat.*, **6**, 779(1963).
(6) Yu, T. F., *Mod. Med.*, **33**, 209(1965).
(7) Smyth, C. J., *Arthritis Rheumat.*, **8**, 907(1965).
(8) DeConti, R., and Calabresi, P., *New Engl. J. Med.*, **274**, 481(1966).
(9) Bartels, E. C., *Ann. Int. Med.*, **64**, 1173(1966).
(10) *J. New Drugs*, **6**, 361(1966).
(11) Zoellner, N., and Schattenkirchner, M., *Deut. Med. Wochschr.*, **92**, 654(1967).
(12) Hall, A. P., Holloway, V. P., and Scott, J. T., *Ann. Rheumat. Dis.*, **23**, 439(1964).
(13) Wyngaarden, J. B., Rundles, R. W., and Metz, E. N., *Ann. Int. Med.*, **62**, 842(1965).
(14) Wyngaarden, J. B., *Arthritis Rheumat.*, **8**, 883(1965).
(15) Houpt, J. B., *ibid.*, **8**, 899(1965).
(16) Goldfarb, E., and Smyth, C. J., *ibid.*, **9**, 414(1966).
(17) Elion, G. B., Callahan, S., Nathan, H., Bieber, S., Rundles, R. W., and Hitchings, G. H., *Biochem. Pharmacol.*, **12**, 85(1963).
(18) Burroughs Wellcome & Co., Brit. pat., 975,850 (Nov. 18, 1964).
(19) Krakoff, I. H., and Meyer, R. L., *J. Pharmacol. Exptl. Therap.*, **149**, 417(1965).
(20) Watts, R. W. E., *Roy. Soc. Med.*, **59**, 287(1966).
(21) Elion, G. B., Kovensky, A., Hitchings, G. H., Metz, E., and Rundles, R. W., *Biochem. Pharmacol.*, **15**, 863(1966).
(22) Sweetman, L., and Nyham, W. L., *Nature*, **215**, 859(1967).
(23) Boni, P., DeLerma, B., and Parisi, G., *Experientia*, **23**, 186(1967).
(24) Krakoff, I. H., *Arthritis Rheumat.*, **8**, 896(1965).
(25) Behrend, R., *Ann.*, **245**, 213(1888).
(26) Behrend, R., and Ernert, P., *ibid.*, **258**, 347(1890).
(27) Shaw, E., and Woolley, D. W., *J. Biol. Chem.*, **194**, 641(1952).
(28) Baker, B. R., and Hendrickson, J. L., *J. Pharm. Sci.*, **56**, 955(1967).
(29) Biesele, J. J., *Cancer*, **5**, 787(1952).
(30) Fjelde, A., *Z. Krebsforsch*, **61**, 364(1956).
(31) Woolley, D. W., and Shaw, E., *J. Biol. Chem.*, **189**, 401(1951).
(32) Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, **128**, 537(1939).
(33) Shealy, Y. F., Struck, R. F., Holum, L. B., and Montgomery, J. A., *J. Org. Chem.*, **26**, 2396(1961).

Keyphrases

Pyrazoles
4-Hydroxypyrazolo [3,4-*d*]-*v*-triazine—synthesis
Bratton-Marshall test—identity
IR spectrophotometry—structure
UV spectrophotometry—structure